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Molecular control of microsporogenesis in *Arabidopsis*

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Microsporogenesis is essential for male fertility and requires both the formation of somatic and reproductive cells in the anther and meiotic segregation of homologous chromosomes. Molecular genetic studies have uncovered signaling molecules and transcription factors that play crucial roles in determining the anther cell types and in controlling gene expression for microsporogenesis. At the same time, key components of in meiotic recombination pathways have been discovered, enriching our knowledge about plant reproductive development.

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Introduction

In flowering plants, the male reproductive organ stamen usually contains four anther lobes, each with a microsporagium where the pollen grains complete their development. Male reproduction has several steps, including the initiation of the stamen from the floral meristem and the generation of the germ-line meiotic cells and somatic cell layers, including the tapetum (Figure 1). These are followed by meiosis and tapetum development that support pollen development, which also requires gametophytic gene functions inside the pollen. This review focuses on recent advances in the understanding of gene functions for early anther development, including the determination of the stamen identity and morphogenesis of the lobed anther structure, the specification of various anther cell layers, the meiotic processes, and anther functions crucial for meiotic cytokinesis and early microspore development. Because of space constraints, the emphasis will be on Arabidopsis genes, with brief discussion of genes from other plants.

Control of stamen identity and the four-lobed structure

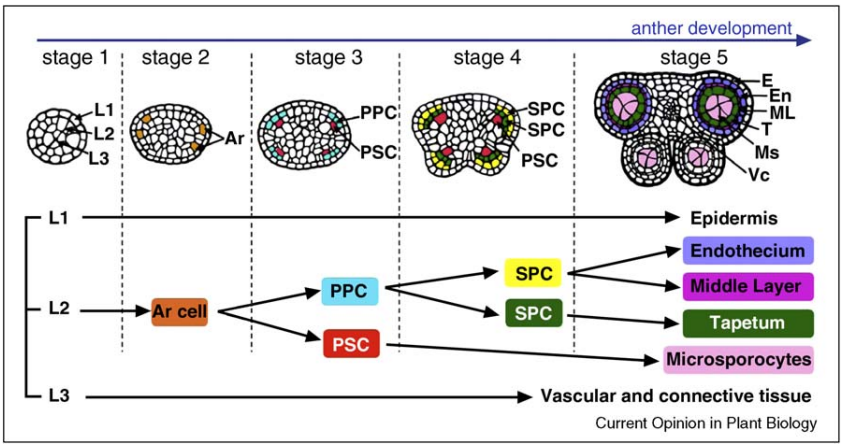
According to the ABCE model for floral organ identities, the combinatorial action of BCE genes determines the stamen identity [1,2]. In Arabidopsis, the stamen requires cooperation of B-class genes *APETELA3* (*AP3*) and *PISTILLATA* (*PI*), C-class gene *AGAMOUS* (*AG*), and at least one of the E-class genes *SEPALLATA1/2/3/4* [1]. In addition, *WUSCHEL* (*WUS*) activates *AG* in the early floral meristem, together with *LEAFY* (*LFY*) [3]. However, in later stages of floral development, *AG* represses *WUS* [4] and promotes the stamen differentiation by cooperating with B and E-class genes, possibly as a heterotetramer [1].

In each anther lobe, cell division and differentiation generate reproductive pollen mother cells (PMCs; or meiotic cells), which are surrounded by four somatic cell layers: the tapetum, the middle layer, endothecium, and the epidermis from the inner to outside (Figure 1). Except the epidermis, which is the result of division of the L1 cells, the three inner somatic cell layers and meiotic cells are generated from the L2 cells called archesporial cells [1,5]. Thus, one of the earliest, possibly most important, events in anther development is the formation of the archesporial cells. Recent studies strongly suggested that the ERECTA (ER) and ER-Like 1 and 2 (ERL1,2) leucine-rich repeat receptor-like protein kinases (LRR-RLKs) are important for anther lobe formation, because the corresponding triple mutant is often defective in the formation of two to four anther lobes and the correct cell patterning within the lobe [6,7]. It is possible that these genes are required for the specification of the archesporial cells; alternatively, they might promote the cell division of archesporial cells to form the multiple cell layers in the lobe. In addition, mutants defective in two cytoplasmic kinases, the MPK3/6 MAP kinases, exhibit similar anther phenotypes to those of the *er erl1 erl2* mutant, suggesting that the MAP kinase cascade might act downstream of the ER/ERL1/ERL2 LRR-RLKs [6]. Further understanding of the functions of ER/ERL1/2 and MPK3/6 will benefit from analysis using archesporial cell-specific markers and genetic dissections of the interactions between these genes.

Cell type specification in the anther

Within each anther lobe, the specification of reproductive and somatic cell types is crucial for early anther development. Acting downstream of the *AG* gene, the *SPOROCYTELESS/NOZZLE* (*SPL/NZZ*) gene is essential for the formation of reproductive cells [8••,9••,10]. *SPL*

Figure 1



Formation of Arabidopsis anther cell layers. The anther primordium only contains the L1, L2, and L3 layers at stage 1. At stage 2, some cells in the L2 layer become archesporial cells, which divide to produce the primary parietal cells (PPC, blue) and the primary sporogenous cells (PSC, red) at stage 3. Then the PPCs divide to form two layers of secondary parietal cells (SPC) at stage 4. Subsequently, the inner SPCs (green) form the tapetum (T, green), and the outer SPC (yellow) divide and differentiate into the middle layer (ML, dark pink) and the endothecium (En, purple). At the same time, the PSCs give rise to the microsporocytes (Ms, light pink) at stage 5.

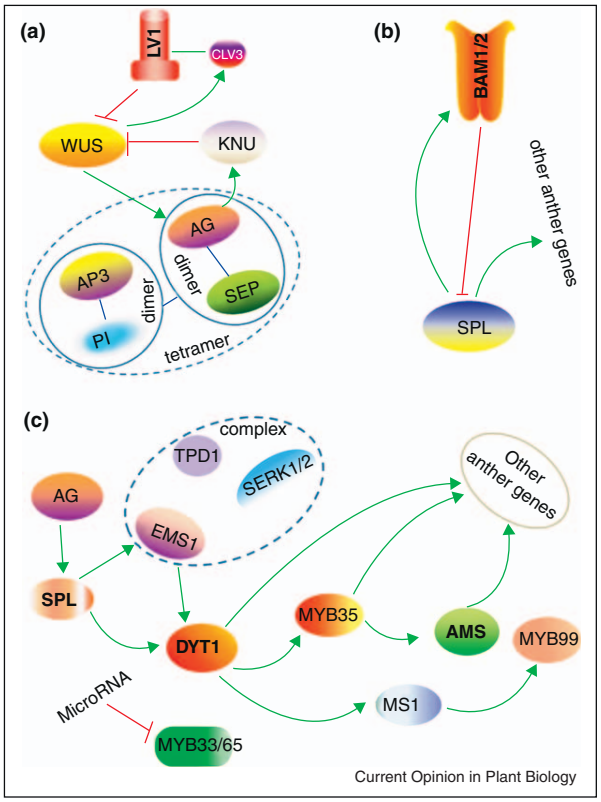
encodes a putative novel transcription factor and is expressed in the L2 layer, becoming restricted to the reproductive precursors as the cell layers are formed, suggesting that it acts in the reproductive cell lineage to specify of their fate [8••]. Conversely, the BARELY ANY MERISTEM1 (BAM1)/BAM2 LRR-RLKs are important for the formation of primary parietal cells (PPC); in the *bam1/2* double mutant anthers, the inner three somatic cell layers are replaced by PMC-like cells [11]. Furthermore, *BAM1/BAM2* expression is reduced in the *spl* mutant, whereas *SPL* expression expands to all subepidermal cells in the *bam1/2* anthers [11]. The functions and interactions between SPL and BAM1/2 can be explained by a positive–negative feedback loop [11] (Figure 2), which controls the balance between the reproductive cell fate and the somatic cell fates during anther development.

Slightly later during anther development, several genes have been found to control the formation and differentiation of the tapetum, one of the three L2-derived somatic cell layers. Among these, the *EXCESS MICROSPOROCYTES1* (*EMS1*)/*EXTRA SPOROGENOUS CELLS* (*EXS*) gene encoding an LRR-RLK is required for the specification of the tapetum fate [12••,13]. The *ems1/exs* mutants produce anthers lacking the tapetum while having extra PMCs. The *EMS1/EXS* gene is expressed in the tapetal precursors and the expression becomes concentrated in developing tapetal cells, suggesting that extracellular signal(s) stimulates the division and differentiation that result in the formation of tapetal cells [12••]. In addition, very similar phenotypes of the absence of tapetal cells with extra PMCs have been observed in the *tpd1* mutant defective in a gene encoding a putative secreted protein, and in the double mutant of the *Somatic Embryogenesis Receptor-like Kinase1* (*SERK1*) and *SERK2* genes encoding LRR-RLKs [14•,15•,16]. These and additional genetic results suggest that TPD1 and SERK1/2 act in the same signaling pathways [16,17]; this idea is further supported by the physical interaction between EMS1/EXS and TPD1 [18]. The highest expression of *EMS1/EXS* is in the nascent tapetum, whereas that of *TPD1* is in the PMCs [12••,16], suggesting that the communication between the PMCs and the tapetal precursors is important for the tapetal fate. It is possible that EMS1/EXS and SERK1/2 form a heteromeric receptor complex and TPD1 serves as the extracellular ligand for the receptor, thereby mediating the specification of tapetum [17–19]. Mutants similar to *ems1/exs* and *tpd1* have also been found in both rice [20,21] and maize [22], suggesting that the cell-cell communication for tapetum specification is conserved in flowering plants.

Regulation of tapetum function and its interaction with meiocytes

Following anther cell specification, subsequent development requires additional gene functions, as revealed by recent studies. *RECEPTOR-LIKE PROTEIN KINASE2* (*RPK2*) is essential for the development of both the tapetum and middle layer in *Arabidopsis*. Unlike the *ems1* mutant that totally lacks the tapetum, the *rpk2* mutant forms an abnormal tapetum and a defective middle layer [23], suggesting that it is involved in cell-cell signaling for both tapetum and middle layer. In addition, the *DYSFUNCTIONAL TAPETUM1* (*DYT1*) gene encoding a bHLH transcription factor is needed for normal tapetum development and function [24•]. The

Figure 2



Genetic interactions during anther development. Summary diagram shows the known genetic interactions between genes during anther development. **(a)** Feedback regulation between CLV and WUS, and between WUS and AG; together they promote the initiation and identity of the stamen; **(b)** The feedback loop between BAM1/2 and SPL, which balances the development of reproductive cells and the somatic cell layers. **(c)** The regulation network among EMS1-SERK1/2 receptor transcription factors, and microRNA, which are crucial for tapetal identity, tapetal function, and pollen development, respectively. The positive regulation is shown by green arrows, the negative regulation by red T-bars, and protein–protein interactions are shown by blue lines (confirmed interaction by solid lines and speculative interaction lacking experimental support by dashed lines). Protein complexes are highlighted by grey circles (putative complex by dashed circles and confirmed complex by solid circles). The arrows, lines, T-bars do not necessarily represent direct interactions, and events in the figures are not all in the same cell.

dyt1 mutant shows abnormal tapetal cells and altered expression of genes important for post-meiotic anther development [24•].

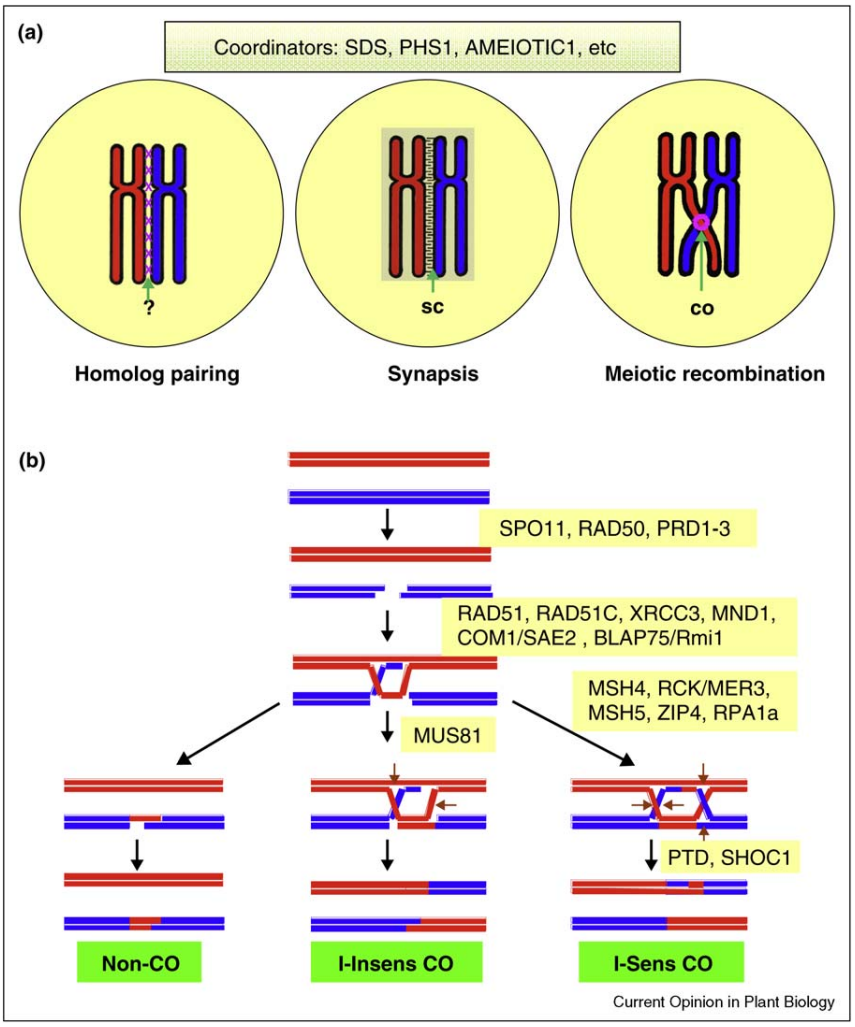
After the formation of reproductive and somatic anther cells, meiosis takes place with the characteristic processes of chromosome condensation and homolog associations. Although the tapetum plays crucial roles in supporting pollen development following the completion of meiosis, it was not known whether the tapetum is also needed for normal meiosis. The mutants such as *ems1* that lack the tapetum provided an opportunity to test for a possible role of the tapetum in supporting meiosis; it was found that meiotic nuclear division in the *ems1* mutant occurred in a way similar to that in the wild-type, producing four nuclei, even in cells occupying the positions of tapetal cells [12••]. Similarly, the *dyt1* mutant anthers contain an abnormal tapetum, yet meiosis can still proceed to the formation of four nuclei [24•]. Therefore, normal tapetal function is not needed for meiotic nuclear division. However, these mutants fail to produce microspores, suggesting that the tapetum is important for the meiotic cytokinesis [12••,24•]. DYT1 regulates several genes with important functions in tapetum and pollen development, including *MYB35* (also called *TDF1*) [24•,25].

Meiotic sister cohesion, pairing and synapsis

The function of the reproductive cells is to produce microspores via meiosis [1,25] (Figure 3). Following DNA replication, replicated sister chromatids associate due to sister cohesion and chromosomes condense to form axial elements. Pairing positions homologous axial elements close to each other, allowing synapsis to occur. Homolog pairing at the DNA level occurs by base-pairing, facilitated by DNA double-stranded breaks (DSBs) and subsequent generation of single strand ends. These events also initiate meiotic homolog recombination, which is closely coupled with synapsis. Recombinational crossover together with sister cohesion maintain homolog association until the transition from metaphase I to anaphase I, thereby ensuring high fidelity chromosome segregation and transmission. Plant meiotic gene functions have been studied extensively in Arabidopsis, rice, maize and other plants and have been reviewed extensively elsewhere [1,26]. Here we only highlight several recent findings on meiosis due to limited space.

In Arabidopsis, mutants defective in sister cohesion also are abnormal in chromosome condensation, pairing and synapsis, suggesting a close interdependency among these processes [27]. In particular, the *SWITCH/DYAD* gene encoding a novel protein is a key regulator of sister cohesion; furthermore, female meiosis in *swi* mutants is converted to a mitosis-like division, suggesting an early regulatory role. In addition, the maize *AMEIOTIC1* (*AM1*) gene is homologous to *SWI/DYAD*; mutant defects indicate that *AM1* is required for multiple meiotic processes, including meiotic gene expression, morphogenesis of the meiotic chromosomes, homolog pairing, synapsis and recombination [28••], supporting the hypothesis that *AM1* (and *SWI*) is needed for the initiation of meiosis. Another maize gene, *POOR HOMOLOGOUS SYNAPSIS1 (PHS1)*, is required to coordinate homolog pairing and synapsis. In *phs1* meiocytes, synapsis occurs often between non-homologous chromosomes, suggesting pairing is defective, allowing non-homologs to become closely positioned and then synapsed [26]. Furthermore, PHS1 controls the entry of RAD50 from cytoplasm to the nucleus and

Figure 3



Homolog interaction during Prophase I and meiosis recombination pathway. **(a)** Three critical processes of homolog pairing, synapsis, and recombination during Prophase I during meiosis. SDS, PHS1 and AMEIOTIC1 have been reported to coordinate between their interactions spatially and temporally. The question mark indicates that relatively little is known about pairing; SC represents the synaptonemal complex; CO represents a chiasma. **(b)** A model for plant recombination pathway(s), which have been partially described in previously [26,27]. Here, we updated several genes involved in different pathways, as indicated by gene names.

affects early recombination in both maize and Arabidopsis [29]. RAD50 is part of a protein complex that generates single strand DNAs from DSBs, allowing pairing-related homology search and recombination between homologs, suggesting that PHS1 controls pairing and recombination by facilitating the import of crucial recombinational proteins into the nucleus [29].

Synapsis requires a number of proteins, including the Arabidopsis ZYP1 protein [27]. Mutants defective in the rice *ZYP1* homolog, *ZEP1*, are abnormal in synapsis; however, the mutant is able to form crossovers, even with an increased number than that in the wild type. This difference from synaptic mutants in Arabidopsis and yeast suggests that *ZEP1* might have distinct functions in rice, especially regarding the coordination between synapsis and CO formation [30].

Meiotic recombination: DSBs formation and repair

An initial step for recombination is the generation of DSBs throughout the genome by SPO11, originally identified in the budding yeast and similar to the A subunit of archaebacterial topoisomerase IV [27]. In Arabidopsis, *SPO11-1* is required for meiotic DSBs, homolog pairing and synapsis [31], with the residues Gly215, Arg222 and Arg223 crucial for the formation of a DNA-binding surface important for meiosis [32]. In addition, a second

Arabidopsis *SPO11* homolog, *SPO11-2* (a different gene from *SPO11-1*), is also required for meiotic DSBs [33,34]. Like *spo11-1*, the *spo11-2* mutant also lacks meiotic DSBs and is defective in bivalent formation, homolog pairing and synapsis [33,34]. Moreover, the *PUTATIVE RECOMBINATION INITIATION DEFECTS1, 2* and *3* (*PRD1-3*) genes are required for SPO11-dependent DSB formation, with mutant phenotypes similar to those of *spo11-1* and *spo11-2* mutants [35,36], but their molecular functions are not known.

Following the SPO11-dependent formation of DSBs, the RAD50-MRE11-containing protein complex is required to generate single strand ends, and RAD51, RAD51C, and XRCC3 are required for repairing the DSBs, presumably via D-loop and other DNA intermediates of recombination [27] (Figure 3). Mutations in these genes fail to repair the DSBs, resulting in chromosome fragmentation detectable during late prophase I and/or later meiotic stages. The Arabidopsis homologs of yeast *MND1* [37,38] and *COM1/SAE2* [39] are also required for repair of SPO-11-dependent DSBs and homolog synapsis. Furthermore, the Arabidopsis homolog of the human and yeast *BLAP75/Rmi1* genes was found to be important for DSBs repair downstream of the RAD51-mediated step, but dispensable for homolog pairing and synapsis [40].

Meiotic recombination: crossover formation

It has been shown that Arabidopsis meiotic crossovers between homologs can be generated via interference-sensitive (I-Sen) and interference-insensitive (I-Ins) pathways [27] (Figure 3). The I-Sen pathway was previously found to require *MSH4*, *MLH3*, *MER3/RCK*, and *PTD* [41,42,43•,44]. In addition, *MSH5*, *ZIP4/SPO22*, *RPA1a* and *SHOC1* are also needed for the I-Sen pathway for CO formation [45,46•,47,48]. Mutations of these genes dramatically reduce the CO frequency. Sequence similarity to yeast genes suggests that MSH4/5 and MER3/RCK promote Holliday junction formation. On the other hand, genetic and phenotypic analysis demonstrated that *PTD* acts downstream of *MSH4* and *MER3/RCK*, probably serving as a Holliday junction resolvase; PTD is slightly similar in sequence to the ERCC1 protein, which forms a heterodimer with XPF for excision repair [43•]. SHOC1 is similar to XPF and required CO formation [46•], suggesting that PTD and SHOC1 form an ERCC1-XPF-like complex to mediate Holliday junction resolution (Figure 3).

In yeast, *MUS81* is required for CO formation via the I-Ins pathway and encodes a structure-specific endonuclease; the Arabidopsis *MUS81* homolog is involved in this pathway [49•,50•], although with less obvious mutant defects. The *mus81* mutant shows no apparent abnormality during meiosis; however, using a fluorescence visual assay and tetrad analysis, the *mus81* mutant was found to have a moderate reduction of meiotic recombination frequency, with the remaining crossovers being interference sensitive [49•]. Furthermore, the *msh4 mus81* double mutant exhibited significantly lower chiasma frequency than that of the *msh4* single mutant. These results support the idea that *MUS81* is part of the I-Ins pathway, not the MSH4-dependent I-Sen pathway, for crossover formation. The fact that the *msh4 mus81* double mutant can still form some COs indicate there is additional pathway(s) for CO formation.

Spindle organization and chromosome segregation

Following prophase I, chromosome separation requires proper spindle function. Previously, the Arabidopsis *ATK1* gene encoding a C-terminal type kinesin motor protein was found to promote normal spindle assembly in male meiosis I and II [51]. More recently, ATK1 and closely related ATK5 (renamed AtKIN14a and AtKIN14b, respectively), were shown to both contribute to spindle assembly during male and female meioses [52]. In addition, *MULTIPOLAR SPINDLE 1* (*MPS1*), is another gene involved in meiotic spindle organization in Arabidopsis. In *msp1* meiocytes, unequal bipolar or multipolar spindles are formed, causing abnormal chromosome segregation and male and female sterility [53].

Tapetum function supporting microsporogenesis

As discussed above, meiotic nuclear division is independent of the tapetum, as seen in *ems1* and *dyt1* mutants; however, the meiotic cell wall is abnormal and meiotic cytokinesis fails to occur in these mutants [12,24•], suggesting that the tapetum is important for normal meiotic cell wall properties and for meiotic cytokinesis. Among the genes that show reduced expression in the *dyt1* mutant, several genes are important for post-meiotic tapetal function [24•], including *ABORTED MICROSPORES* (*AMS*), *MALE STERILITY1* (*MS1*), *MYB35/TDF1, and MYB103/80* [25,54,55,56•]. AMS is also a bHLH protein and is required for normal microspore development [54] and regulates the expression of many floral genes [55]. *AMS* is positively regulated by the transcription factor MYB35, which is downstream of DYT1 [24•,25]. *MS1* encodes a PHD transcription factor, and is required for tapetum gene expression supporting pollen wall formation [56•]. Furthermore, *ms1* mutant anther displays mis-expression of numerous anther genes, including *AtMYB99* [57•]. Furthermore, transcriptional regulation is likely conserved homologs of Arabidopsis *DYT1*, *AMS*, and several MYB genes have been shown to have similar functions in rice [58–62].

In summary, much progress has been made in recent years in the understanding of genes for both early anther development and meiosis, both important for microsporogenesis. Future studies will benefit from technological

advances and comparative analyses and will not only deepen the understanding in model systems, but also broaden the knowledge to other plants, including crops, thereby promoting plant breeding and other applications.

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